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## Critical Photoperiod for Flower Bud Initiation in Five Everbearing Strawberry Cultivars

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### Summary

Five everbearing strawberry (*Fragaria × ananassa* Duch.) cultivars, 'Rabunda', 'Appelever', 'Natsuakari', 'Deco-Rouge', and 'Morioka No. 26', were grown at day (6:00–18:00)/night (18:00–6:00) temperatures of 30/25°C. Plants were grown under 13-, 14-, 15-, and 16-hr photoperiods for 16 weeks ('Natsuakari' and 'Morioka No. 26') or 24 weeks ('Deco-Rouge'). In addition to these photoperiods, 11- and 12-hr photoperiods were set for 'Rabunda' and 'Appelever', and 'Rabunda' was grown under these photoperiods for 20 weeks and 'Appelever' for 24 weeks. Under such a high-temperature condition, 'Appelever' was found to be a quantitative long-day plant, while 'Natsuakari', 'Deco-Rouge', and 'Morioka No. 26' were qualitative long-day plants. Inflorescence production in 'Rabunda' was low throughout the treatment under all photoperiods. After the photoperiod treatment, plants were dissected under a microscope to observe the presence of flower buds. We found no flower primordia in 'Rabunda' plants under the 16-hr and shorter photoperiods, in 'Natsuakari' plants under the 14-hr or shorter photoperiods, or in 'Deco-Rouge' or 'Morioka No. 26' plants under the 13-hr photoperiod. Moreover, in 'Morioka No. 26', seven of ten plants had not initiated flower primordia under the 14-hr photoperiod, although the others had initiated flower buds. We conclude that the critical photoperiod for flower bud initiation in 'Morioka No. 26' may exist between the 13- and 15-hr photoperiod. Flower bud initiation in 'Appelever' was not inhibited, so its critical photoperiod may be shorter than 11 hr. The critical photoperiod for flower bud initiation in everbearing strawberry plants grown at high temperature varies among cultivars.

Key words: everbearing strawberry, high temperature, reproductive growth, vegetative growth

Based on the temperature and photoperiodic requirements for flower bud initiation, cultivated strawberry (*Fragaria × ananassa* Duch.) plants are categorized as June-bearing, everbearing, or day-neutral cultivars. In Japan, most strawberry cultivars grown for fruit production are June-bearing, single-cropping type. June-bearing strawberry cultivars are classified as short-day plants.

Unlike June-bearing strawberry cultivars, everbearing strawberry cultivars are suitable for summer cropping, as they can initiate flower buds and produce fruits even in summer under natural conditions. Everbearing strawberry cultivars are classified as long-day plants, and they produce more inflorescences under longer photoperiods at less than 26–27°C (Durner *et al.*, 1984; Sønsteby and Heide, 2007a; Smeets, 1980; Taimatsu, 1993). In previous reports we also indicated that ‘Summerberry’, an everbearing strawberry cultivar, is a quantitative long-day plant, because the cultivar produced inflorescence continuously under either an 8-hr or a 24-hr photoperiod, although more inflorescences were produced under the 24-hr photoperiod than under the 8-hr photoperiod at day (6:00–18:00)/night (18:00–6:00) temperatures of 20/15°C and 25/20°C. At 30/25°C, however, flower bud initiation and inflorescence production is inhibited under an 8-hr photoperiod, although it is remarkably promoted under a 24-hr photoperiod. This indicates that ‘Summerberry’ is a qualitative long-day plant under high temperature (Nishiyama *et al.*, 1998, 1999). A similar result was reported recently by Sønsteby and Heide (2007b). Critical photoperiod is defined as the longest photoperiod at which the plant will flower, or beyond which the plant will not flower. So we decided to use the term “critical photoperiod” to indicate the difference between when flower bud initiation is inhibited or promoted.

Nishiyama *et al.* (2006) investigated the precise critical photoperiod for flower bud initiation in ‘Summerberry’ at 30/25°C and found it was between 13- and 14-hrs. Other everbearing strawberry cultivars were also cultivated under high temperature and various photoperiods, and the results confirmed that each cultivar has a critical photoperiod and that the critical photoperiod varies among cultivars (Nishiyama *et al.*, 2009). Day-neutral strawberry cultivars, whose flowering habits are derived from *F. virginia* ssp. *glauca* (Wats.) Staudt and that produce inflorescence constantly regardless of photoperiod (Dunner *et al.*, 1984; Manakasem and Goodwin, 2001), were also tested, and a similar result like that for everbearing strawberry cultivars was obtained (Nishiyama *et al.*, 2007).

Flower bud initiation in everbearing strawberry cultivars under cultivation was reported to be inhibited under high temperature (Oda and Yanagi 1990; Taimatsu, 1993). On the other hand, it was also reported that flower bud initiation was strongly promoted and axillary bud initiation was inhibited under illuminated cultivation in summer (Kono and Tsunematsu, 1993, 1994). Based on our results, we believe these conflicting findings can be explained as follows: an inhibition of flower bud in summer is caused by a combination of high temperature and a daylength that is shorter than the critical photoperiod, and a promotion of flower bud initiation is caused by a combination of high temperature and a daylength that is longer than the critical photoperiod.

As mentioned above, the critical photoperiod in everbearing strawberry cultivars seems an important feature in hot summer cultivation, so we decided to

investigate the critical photoperiods for five cultivars including two newly registered cultivars and one of parent cultivars in this study.

## Materials and Methods

### *Plant Materials*

Five everbearing strawberry (*Fragaria* × *ananassa* Duch.) plants, 'Rabunda' (bred in Netherlands and released in 1969), 'Appelever' (bred in France), 'Natsuakari' (everbearing strawberry 'Summerberry' × June-bearing strawberry 'Kita-nokagayaki', registered in 2007, line name 'Morioka No. 29'), 'Deco-Rouge' (intermediate type strawberry 'Pajaro' × everbearing strawberry 'Morioka No. 26', registered in 2007, line name 'Morioka No. 31'), and 'Morioka No. 26', were used in this experiment.

### *Cultivation and Photoperiod Treatment*

The outline of cultivation for each cultivar is shown in Table 1. All cultivars were cultivated by the same method. All plants were potted in 18-cm-diameter clay pots filled with mixed soil (compost : vermiculite : manure = 5 : 3 : 2,

Table 1. Outlines of the cultivations.

Cultivars	Runner plants development <sup>z</sup>	Potting <sup>y</sup>	Transferred <sup>x</sup>	Treatment started <sup>w</sup>
Rabunda	Summer in '01	26 Nov. '01	7 May '02	16 May '02
Appelever	Summer in '02	30 Aug.-4 Sep. '02	18 Oct. '02	24 Oct. '02
Natsuakari	Summer in '04	Jul. '04	9 Sep. '04	16 Sep. '04
Deco-Rouge	Summer in '05	15-16 Aug. '05	13 Oct. '05	20 Oct. '05
Morioka No. 26	Summer in '06	15, 17 Nov. '06	22 Feb. '07	8 Mar. '07

<sup>z</sup> 'Rabunda', 'Appelever', and 'Morioka No. 26' plants were propagated in the nursery field of Tohoku university. 'Natsuakari' and 'Deco-Rouge' plants were obtained from National Agricultural Research Center for Tohoku Region. All plants were grown under a nursery field until potting.

<sup>y</sup> After potting, plants were grown in an unheated, natural daylength glasshouse ('Rabunda' and 'Morioka No. 26') or in the nursery field ('Appelever', 'Natsuakari', and 'Deco-Rouge').

<sup>x</sup> Plants were transferred into the phytotrons under natural daylength at this date and were acclimated until treatments started. Acclimation conditions were as follows; 'Rabunda' were treated at day (6:00-18:00)/night (18:00-6:00) temperature of 25/20°C for nine days. 'Appelever' at 20/15°C for six days, 'Natsuakari' and 'Deco-Rouge' at 25/20°C for seven days, and 'Morioka No. 26' at 20/25°C for seven days followed by at 25/20°C for seven days.

<sup>w</sup> From this date, high temperature and photoperiod treatment started. Plants were grown in the phytotrons controlled at 30/25°C under various photoperiods. For the photoperiods, see Table 2.

v/v/v). During the potting, 3 g of compound fertilizer ( $N : P_2O_5 : K_2O = 15 : 15 : 15$ ) was mixed into the soil in each pot, and an additional 1 g of the fertilizer was applied every two weeks after the start of the treatment. Plants were irrigated once a day.

After potting, plants were grown in an unheated, natural daylength glass-house ('Rabunda' and 'Morioka No. 26') or in the nursery field ('Appelever', 'Natsuakari', and 'Deco-Rouge'). All plants were transferred to natural daylight type phytotrons for the temperature and photoperiod treatments. Dates of transfer are shown in Table 1. Before high temperature and photoperiod treatment, plants were acclimated under natural daylight. 'Rabunda' was acclimated at day (6:00–18:00)/night (18:00–6:00) temperatures of 25/20°C for nine days, 'Appelever' at 20/15°C for six days, 'Natsuakari' and 'Deco-Rouge' at 25/20°C for seven days, and 'Morioka No. 26' at 20/25°C for seven days followed by 25/20°C for seven days.

All cultivars were grown under 13-, 14-, 15-, and 16-hr photoperiods. In addition to these photoperiods, 11-, and 12-hr photoperiods were set for 'Rabunda' and 'Appelever'. All plants were grown under natural daylight from 9:00 to 17:00 and were covered with double silver polyethylene film from 17:00 to the following 9:00. Supplemental light was provided with 100-W incandescent lamps that irradiated 26.0–36.5  $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  light intensity at the top of the plants. These supplemental light periods were from 7:30 to 9:00 and 17:00 to 18:30 for the 11-hr, 7:00 to 9:00 and 17:00 to 19:00 for the 12-hr, 6:30 to 9:00 and 17:00 to 19:30 for the 13-hr, 6:00 to 9:00 and 17:00 to 20:00 for the 14-hr, 5:30 to 9:00 and 17:00 to 20:30 for the 15-hr, and 5:00 to 9:00 and 17:00 to 21:00 for the 16-hr photoperiods. 'Rabunda' plants were treated for 20 weeks, 'Appelever' and 'Deco-Rouge' plants were treated for 24 weeks, and 'Natsuakari' and 'Morioka No. 26' plants were treated for 16 weeks.

#### *Measurement Methods*

At the start of the high temperature and photoperiod treatment, each plant had a few branch crowns, which we removed so as to leave one branch crown per plant. The remaining branch crown was regarded as the main shoot. The apices of removed branch crowns were dissected under a stereoscopic microscope, and flower buds were observed in all apices irrespective of cultivars.

Once a week during this period, the number of inflorescences with one or more open flowers, expanded leaves, generated runners, and branch crowns was counted, although data on runners and branch crowns are not shown in this report. The counted inflorescences and runners were removed. To measure the petiole length, we cut off the leaves 4–6 weeks after their expansion, although data on petiole length are not shown in this report. Average numbers of counted leaves and inflorescences per plant were summed every 4 weeks and are shown in Figure 1 and

2, respectively. Counted inflorescences and runners were cut off. During strawberry cultivation it is usual to leave one or two branch crowns and remove the rest; in this experiment we left all branch crowns. Seven plants of 'Rabunda', ten 'Appelever' and 'Natsuakari', twelve 'Deco-Rouge', and eleven 'Morioka No. 26' plants were grown in each photoperiod treatment. A few plants became wilted during the treatment, and these wilted plants were excluded from the data. Table 2 shows the number of plants observed in each treatment.

### Results

The numbers of leaves that expanded during the treatment were shown in Table 2. They were significant among the photoperiod treatments in 'Appelever' and 'Morioka No. 26'. The changes in the numbers of expanded leaves per plant during the photoperiod treatment are shown in Fig. 1. Although there were significant differences among the photoperiod treatments in some cultivars, leaves expanded continuously throughout the treatment in all cultivars irrespective of photoperiod. Leaves expanded almost constantly under the 11- and 12-hr in 'Appelever' and under the 13-hr in 'Morioka No. 26'. On the other hand, the number of expanded leaves in these cultivars under the longer photoperiod treatments increased during the latter half of the treatment.

The numbers of inflorescences that flowered during the treatment were shown in Table 2. Those of 'Rabunda' were not significant among photoperiods. Those of 'Appelever' were significantly higher under the longer photoperiods. Those of 'Natsuakari' were higher under the 16-hr photoperiods than under the 15-hr or shorter photoperiods. Those of 'Deco-Rouge' were significant higher under the 14-, 15-, and 16-hr photoperiods than under the 13-hr photoperiod. Those of 'Morioka No. 26' were the highest under the 15- and 16-hr photoperiods, followed by the 14-hr photoperiod, and they were the lowest under the 13-hr photoperiod. The changes in the numbers of inflorescences per plant during the photoperiod treatment are shown in Fig. 2. Inflorescence production continued throughout the treatment under all photoperiods in 'Appelever'. Those in other cultivars stopped by the end of the treatment under some photoperiod. The photoperiods under which inflorescence productions stopped were the 16-hr or shorter photoperiod in 'Rabunda', the 13- and 14-hr photoperiod in 'Natsuakari', and the 13-hr photoperiod in 'Deco-Rouge' and 'Morioka No. 26'.

After the photoperiod treatment, plants were dissected under a microscope to observe the presence of flower buds. We found no flower primordia in 'Rabunda' plants under the 16-hr or shorter photoperiods, in 'Natsuakari' plants under the 13- and 14-hr photoperiods, or in 'Deco-Rouge' and 'Morioka No. 26' plants under the 13-hr photoperiod. Moreover, in 'Morioka No. 26', seven of ten plants had not initiated flower primordia under the 14-hr photoperiod, although the others had

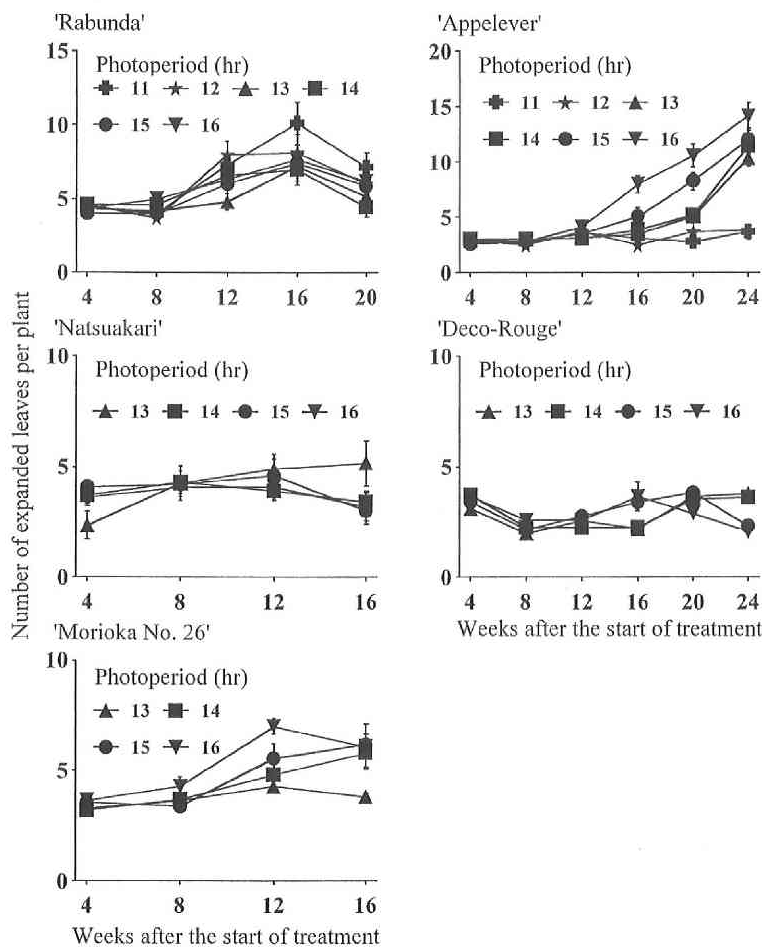


FIG. 1. Effect of photoperiod on the number of expanded leaves per plant grown at 30/25°C. Data are shown as 4-week averages. Vertical bars represent SE. The outlines of the cultivation conditions for each cultivar are shown in Table 1.

initiated flower buds.

These results showed that the critical photoperiods for flower bud initiation at 30/25°C exist between the 14- and 15-hr photoperiod in 'Natsuakari', between the 13- and 14-hr photoperiod in 'Deco-Rouge', and between the 13- and 15-hr photoperiod in 'Morioka No. 26'. That of 'Rabunda' is expected to be longer than 16-hr, and that in 'Appelver' is expected to be shorter than 11-hr.

### Discussion

As shown in previous reports (Nishiyama *et al.*, 2006; Nishiyama *et al.*,

Table 2. Effect of photoperiod on the growth of everbearing strawberry cultivars grown at high temperature<sup>z</sup>.

Photoperiod (hr)	Number of plants observed	Number per plant	
		Expanded leaves	Inflorescences
Rubunda			
11	7	33.0 a <sup>y</sup>	3.4 a
12	7	30.4 a	3.4 a
13	6	25.7 a	2.8 a
14	7	27.1 a	2.3 a
15	7	27.3 a	2.6 a
16	6	29.5 a	3.2 a
Appelever			
11	9	19.3 C	6.0 C
12	10	19.1 C	5.1 C
13	10	28.0 B	9.0 B
14	10	29.7 B	9.7 B
15	10	34.4 AB	12.2 AB
16	10	42.5 A	18.5 A
Natsuakari			
13	10	16.7 A	0.3 B
14	10	15.3 A	0.3 B
15	10	15.9 A	2.4 AB
16	10	15.0 A	5.2 A
Deco-Rouge			
13	9	17.3 a	1.0 B
14	11	17.7 a	3.7 A
15	12	17.9 a	6.4 A
16	12	17.5 a	8.4 A
Morioka No. 26			
13	11	15.0 B	1.5 C
14	10	17.5 AB	3.2 B
15	11	18.6 A	7.5 A
16	11	21.0 A	9.5 A

<sup>z</sup> Plants were grown at a day (6:00-18:00)/night (18:00-6:00) temperature of 30/25°C for 20 weeks ('Rubunda'), 24 weeks ('Appelever' and 'Deco-Rouge'), or 16 weeks ('Natsuakari' and 'Morioka No. 26'). The outlines of the cultivations for each cultivar are shown in Table 1.

<sup>y</sup> Different letters within cultivars and columns indicate significance at  $P=0.05$  by Tukey-Kramer multiple comparisons test (small letters) or Steel-Dwass test (capital letters).



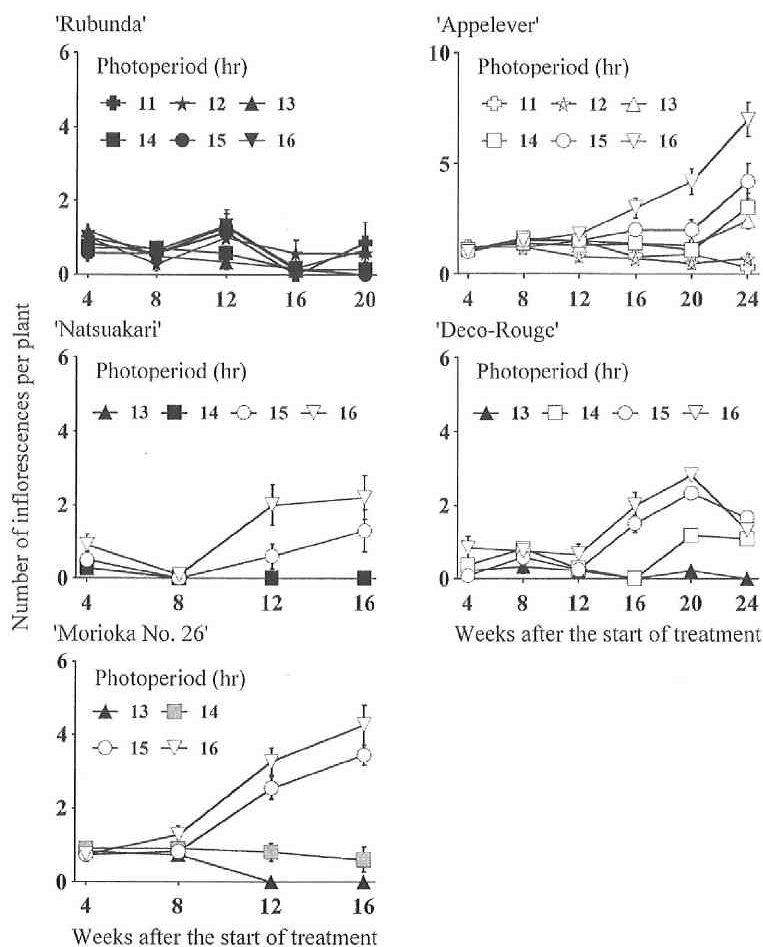


FIG. 2. Effect of photoperiod on the number of inflorescences per plant grown at 30/25°C. Data are shown as 4-week averages. Vertical bars represent SE. Solid symbols: After the high temperature and photoperiod treatment, plants were dissected under a microscope to observe the presence of flower buds. Plants had not initiated flower primordia under these photoperiods. Open symbols: Inflorescence production continued throughout the test period under these photoperiods. Gray symbols: A few plants had flower primordia at the microscopic observation. The outlines of the cultivation conditions for each cultivar are shown in Table 1.

2009), the five everbearing strawberry cultivars used in this experiment were also qualitative long-day plants when these cultivars were grown under high temperature such as 30/25°C. These earlier studies also showed that the critical photoperiod varies among cultivars. Although flower bud initiation was inhibited in 'Rubunda' under all photoperiods tested in this experiment, this cultivar was reported as a long-day plant (Smeets, 1980). Smeets reported that the percentage

of flowering plants was higher under the 24-hr than the 8- or 16-hr photoperiod at 26°C. In our study, temperature was a little higher than that used in the study of Smeets (1980), and we expected that photoperiods longer than 16-hr would induce flower bud initiation at 30/25°C. Under the 14-hr photoperiod, although the number of inflorescence was less than one per plant per 4-week period, inflorescence production continued until the end of treatment in 'Morioka No. 26'. Flower buds in these plants had initiated by the time plants were dissected after the treatment. Because of this, we concluded that the critical photoperiod of 'Morioka No. 26' was probably between the 13- and 15-hr photoperiod used in the present experiment, though more investigation is needed to clarify the exact time that would comprise the critical photoperiod for this cultivar.

There are a few reports on the effects of temperature and/or photoperiod on flower bud initiation in 'Natsuakari' and 'Deco-Rouge', although one study showed that the percentage of flowering plants was higher under the 16-hr photoperiod than under the 8-hr photoperiod at 20°C (Hamano *et al.*, 2004). This result indicates that these cultivars are quantitative long-day plants under intermediate temperature. The critical photoperiod in everbearing strawberry cultivars is shown only when plants are grown under high temperature.

Day-neutral strawberry cultivars also have a critical photoperiod (Nishiyama *et al.*, 2007). The results of tests on everbearing and day-neutral strawberry cultivars indicate that they are qualitative long-day plants that cannot be distinguished from one another when they are cultivated under high temperature. The critical photoperiod will be an index for classification (Table 3). The index will be useful in summer cultivation; to initiate flower buds, it is necessary to

Table 3. Differences in critical photoperiod for flower bud initiation among everbearing and day-neutral strawberry cultivars.

Critical Photoperiods	Cultivars
Shorter than 11-hr	'Appelever'
Between 12- and 13-hr	'Miyoshi' <sup>z</sup> , 'Hecker' <sup>y</sup>
Between 13- and 14-hr	'Summerberry' <sup>x</sup> , 'Ohishi-shikinari' <sup>z</sup> , 'Deco-Rouge', 'Aptos' <sup>y</sup> , 'Selva' <sup>y</sup>
Between 14- and 15-hr	'Kaho' <sup>z</sup> , 'Natsuakari'
Between 15- and 16-hr	'Yamato-shikinari' <sup>z</sup>
Between 15- and 24-hr	'Brighton' <sup>y</sup>
Between 16- and 24-hr	'Rabunda'

<sup>z</sup> Nishiyama *et al.* (2009).

<sup>y</sup> Day-neutral cultivars. Nishiyama *et al.* (2007).

<sup>x</sup> Nishiyama and Kanahama (2006).

cultivate plants under longer daylength than the critical photoperiod or to cultivate cultivars whose critical photoperiod is shorter than natural daylength.

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